Trichinellosis during pregnancy: A case control study in the Lao Peoples’ Democratic Republic

T. Taybouavone\textsuperscript{a}, T.N. Hai\textsuperscript{a}, P. Odermatt\textsuperscript{b}, V. Keoluangkhot\textsuperscript{a}, N. Delanos-Gregoire\textsuperscript{c}, J. Dupouy-Camet\textsuperscript{c}, M. Strobel\textsuperscript{a}, H. Barennes\textsuperscript{a,}\textsuperscript{*}

\textsuperscript{a}Institut Francophone pour la Médecine Tropicale, Lao PDR, BP 9519 Vientiane, Lao Democratic People’s Republic
\textsuperscript{b}Swiss Tropical Institute, Basel, Switzerland
\textsuperscript{c}Service de Parasitologie-Mycologie and Centre National de Référence des Trichinella Hôpital Cochin, Paris, France

1. Introduction

Human trichinellosis, a consequence of infection with \textit{Trichinella} spp. parasites, may be a mild or asymptomatic disease but serious disease and fatal outcomes can also occur. It remains a global public health concern, particularly in areas where meat may be consumed raw or undercooked, where pigs are raised outdoors or in contact with wild animals, and where meat inspection is not performed or not performed properly. In many Asian countries such as Laos, Thailand and China, human trichinellosis is frequent, yet often undiagnosed and hence underreported (Pozio and Zarlenga, 2005; Wang et al., 2006; Sayasone et al., 2007; Barennes et al., 2008).

After ingestion of \textit{Trichinella} larvae in contaminated meat, adult worms develop in the small intestine (enteral phase). After a short maturation period, adult female worms release newborn larvae into the blood stream; these newborn larvae migrate to muscles and other organs (parenteral phase). Since the beginning of the last century, it was suspected that vertical transmission of \textit{Trichinella} might occur in pregnant women during the parenteral phase of the infection, or postnatally during lactation. Salzer (1916) reported the presence of \textit{Trichinella} larvae in the mammary glands and milk of a nursing woman who was diagnosed with trichinellosis. Kuitunen-Ekbaum (1941) found larvae in a human fetus and Bourne (1952) reported \textit{Trichinella} cysts in a 6-week-old aborted fetus. Nunez et al. (2002) demonstrated that during pregnancy in...
rats there is an enhanced helminthotoxic effect directed against the newborn larvae and that despite this immunoreactivity, vertical transmission of the parasite was possible.

Vertical transmission has also been documented in ferrets, guinea pigs, and mice (Webster and Kapel, 2005), however, in humans, only one possible case of congenital trichinellosis has been reported (Dubinsky et al., 2001). The importance of vertical transmission of trichinellosis in humans remains an open question as it is rarely addressed in highly endemic settings or during outbreaks. Laos is one of the poorest nations in Southeast Asia. Throughout the country pigs are an important source of food and income for subsistence farmers. Husbandry practices vary between villages. Older pigs may be penned or tethered although it is also common for pigs to roam freely in the village. There is a strong cultural habit of eating raw or undercooked pork. In the aftermath of a large-scale outbreak of trichinellosis in Northern Lao PDR, where more than 650 persons were affected, we tried to assess evidence of vertical transmission of *Trichinella* infection.

2. Methods and population

2.1. Study area

A trichinellosis outbreak with subsequent ongoing transmission occurred between June 2005 and March 2006 in Udomxay Province of Northern Laos. A full account of the outbreak investigation and a description of the outbreak site are given elsewhere (Barennes et al., 2008).

2.2. Identification of mothers and children

We identified women who were pregnant during the outbreak period (May to December 2006) in Udomxay and surrounding villages by reviewing hospital and health center records and by visiting key informants such as the village leaders. Mothers were classified as (i) control: mothers who did not eat raw or undercooked food, did not participate in any outbreak event, and had no clinical signs; (ii) suspect: reported participating in outbreak ceremonies or eating raw pork; (iii) confirmed: met the criteria for suspect and had a history of symptoms of trichinellosis (fever, myalgia, facial edema, and peripheral blood eosinophilia >1000 cells/mm$^3$), and a positive serology test result for *Trichinella* antibodies (ELISA or Western-blot).

We used a structured questionnaire of 45 items to assess household economic status, past episodes of illness, dietary habits, and food intake of mother and child. The mothers were interviewed by a Lao investigator (TT) in the Lao language. We performed clinical examinations on all mothers and children. Blood cell counts were carried out and serology (Western blot) was performed on pregnant women and their children. The investigation of children included a 13-item questionnaire, a physical examination, anthropometric measurements, and a psycho-motor examination using a 6-item scale adapted to infants. Children were classified as follows: (i) unlikely; (ii) suspected: mother suspected, no clinical signs; (iii) possible: mother suspected and clinical signs; and (iv) confirmed case of congenital trichinellosis (different Western blot profiles between the mother and the child). This definition of a confirmed case of congenital infection was defined arbitrarily as we postulated that IgG antibodies which are present in an infant’s serum sample(s) but absent in the mother’s serum sample were generated by the newborn infant as a result of prenatally acquired infection. This is a well-established method for congenital toxoplasmosis where different western-blot profiles are observed between mothers and children (Robert-Gangneux et al., 1999). WHO tables were used to calculate z-scores for each child in height for age (stunting), weight for age (underweight), and weight for height (wasting). Body mass index (BMI) was calculated for mothers (WHO, 2004).

2.3. Laboratory procedures

Serum was collected on No. 4 Whatman filter paper from each mother and child pair, and was sent to the French National Reference Center for *Trichinella* (NRCT), Hospital Cochin, Paris for serological confirmation by Western blot (WB) analysis. A commercially available *Trichinella* Western blot kit (LDBio Diagnostics, Lyons, France) (specificity 99%) was used as previously described (Barennes et al., 2008).

2.4. Data management and analysis

Using Epidata (www.epidata.dk, Odense, Denmark) and Stata, V. 8 (Stata Cooperation, College Station, TX, USA) for data processing. We used Fisher's exact test for categorical variables, Student's t-test for normally distributed continuous variables, a Mac-Nemar test and conditional logistic regression for village matched comparison between cases and controls. We considered *p* < 0.05 to be significant.

Ethical approval for the study was obtained from the Medical Council of the Lao Ministry of Health. Written informed consent was obtained from the mothers.

3. Results

Of 200 pregnant women, 14 were suspected of having been infected with *Trichinella* (Fig. 1). Eight had a positive serology of whom 4 had clinical signs of trichinellosis and could be considered as confirmed cases. Ten women were classified as suspected since they had no symptoms, or, in the case of 4 of these, an isolated positive serology. One of the 4 confirmed mothers underwent a passive abortion at 26 weeks gestation. She complained of cephalgia and rash and received 7 days of treatment with albendazole at 24 weeks gestation. One week later, she developed hepatitis and aborted.

Among children born from infected mothers, one was diagnosed with congenital inter-ventricular cardiac communication. Of 13 children tested, 12 had no anti-*Trichinella* antibodies by Western blot; one child had a positive Western blot for *Trichinella* antibodies and a comparison of the Western blot profile with that of the mother showed a similar pattern suggesting antibodies were present as a result of passive transfer. No subsequent
analysis could be performed to check for possible changes in antibodies over time.

Of the 14 suspected mothers three attended the main ceremonies during the outbreaks; others were exposed to pork meals at other times. The main characteristics of confirmed cases and controls are shown in Table 1. Notably, infected mothers ate raw pork more frequently compared with controls ($p = 0.01$). Multivariate analysis showed that confirmed cases of trichinellosis in mothers were significantly associated with 3 main factors: consumption of raw food during pregnancy (OR: 51, 95% CI: 3–702), having a relative with trichinellosis (OR: 91, 95% CI: 7–1132), and income below 80 USD/month (OR: 0.03, 95% CI: 0.0–0.7). Children born to infected mothers did not clinically differ from controls; none had decreased psycho-motor development or lower birth weights.

4. Discussion

Of 200 women who were pregnant at the time of the outbreak, we suspected 14 cases (7.0%) of *Trichinella* infection, and subsequently confirmed 8 cases. Three of the confirmed cases attended the main ceremonies which was the main source of the large-scale outbreak. Five confirmed cases were infected by eating raw or undercooked pork at other times. During pregnancy and postpartum, Lao women avoid exposure to what they consider to be health risks (Barennes et al., 2007). This includes participation in large meetings and eating raw food.

In the present study, only 2 women with confirmed trichinellosis reported receiving treatment. This behavior may explain the lower than expected number of exposed women, thus, limiting the power of the study. Of the 8 confirmed cases, one (12.5%) experienced a spontaneous abortion at 26 weeks of gestation and another had a child with a non-specific cardiac malformation. No antibodies to *Trichinella* antigens were detected by Western blot in 12/13 children tested; one child had a positive Western blot, but a comparison with the Western blot profile of the mother suggested it was due to passive IgG transfer as has been reported for toxoplasmosis (Robert-Gangneux et al., 1999). All children, except the one with the non-specific malformation, were healthy.

This study underlines the risk for abortion during pregnancy in *Trichinella* infected women as previously reported (Dupouy-Camet et al., 2002) and highlights the need for adopting safe dietary behavior during pregnancy. However, we could not strictly relate the only abortion observed in the study to trichinellosis since hepatitis may have been the cause. The study also shows that half of the mothers with confirmed trichinellosis did not present with clinical symptoms. This could be related to a low number...
of larvae ingested or possibly due to better tolerance of infection during pregnancy.

Acknowledgments

We thank the staff of Udomxay Provincial Health, Veterinarian Departments, Epidemiology Centre, NCRT staff, Dr. B. Martinez-Aussel, Dr. P. Naphayvong, Dr J.P René, Dr P. Vongphrachanh, and IFMT staff. We gratefully thank the populations of the involved villages. We gratefully thank Dr Cindy Chu for revisions. This study was funded by the ‘Agence Universitaire de la Francophonie’ and ‘Coopération pour la Recherche Universitaire et Scientifique’ (CORUS project number 02-811-052), Ministry of Foreign Affairs, France.

Conflict of interest

None declared.

References


